Fish Physiology and Biochemistry **23:** 23–34, 2000.

The response of coral trout (*Plectropomus leopardus*) to capture, handling and transport and shallow water stress

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Accepted: May 19, 2000

Key words: capture and transport, coral trout, immune function, shallow water, stress

Abstract

The stress response of coral trout (*Plectropomus leopardus*) to wild capture or controlled shallow water stressors was investigated. Stress associated with capture from the wild, handling and transport and shallow water evoked significant changes in circulating levels of cortisol, glucose, lactate, haemoglobin (Hb) and haematocrit (Hct) in coral trout. Plasma glucose, Hb and Hct increased over 30 to 60 min in response to the controlled stressor and then returned toward unstressed levels, even if the stressor persisted. Cortisol increased to maximum levels over the 60 min following the onset of the stressor and remained elevated for 4 h in the case of a single 30 min shallow water event or 3 d in response to the capture, handling and transport. The difference between the responses of blood glucose and cortisol suggests that cortisol does not maintain blood glucose during stress in coral trout. The concentrations of circulating thrombocytes, lymphocytes and granulocytes were also significantly affected by shallow water stress. As lymphocytes and granulocytes are important immune components, reduced concentrations of these cells may explain the increases in disease susceptibility, commonly observed in stressed fish during live transport.

Introduction

The concept of biological stress applied to fishes has attracted considerable attention in recent years. While many of the earlier studies addressed the effects of stress on cold water fishes such as salmon (*Oncorhynchus* spp.) and trout (*Salmo* spp.) (e.g., Barton and Peter 1982; Schreck 1982), more recent studies have investigated subtropical species such as snapper (*Pagrus auratus*) (Lowe et al. 1993), red drum (*Sciaenops ocellatus*) (Robertson et al. 1988) and sea bream (*Sparus aurata*) (Arends et al. 1999). Generally, these investigations have sought to provide information aimed at enhancing aquaculture production or explaining fish responses during capture and transport (Carragher and Rees 1994; Davis and Parker 1986).

The response of fish to stress can be divided into primary, secondary and tertiary components, according to the level of organisation at which it operates (Wedemeyer and McLeay 1981; Wendelaar Bonga 1997). The primary response to stress involves the activation of two major systems: the hypothalamic-pituitary-interrenal (HPI) axis and the sympathetico-chromaffin (SC) system. Stimulation of the HPI axis results in increased circulating levels of cortisol while stimulation of the SC system results in increased circulating levels of adrenaline. These neuroendocrine reactions in turn stimulate secondary responses which are manifested as changes in a range of biochemical, physiological, haematological and immunological parameters (Barton and Iwama 1991). If the stress is severe or prolonged, tertiary responses follow. These include alterations in disease resistance, growth, reproductive output, and overall condition and market quality (Lowe et al. 1993; Sumpter 1991).

In general, the magnitude of the stress response reflects the intensity of stress experienced (Barton and Iwama 1991). Accordingly, periodic, quantitative measurements of primary, secondary and tertiary responses allow the intensity of stress experienced by a

fish and the likely effect of that stress on its subsequent health and survival to be evaluated.

Coral trout (*Plectropomus leopardus*: Family Serranidae) is a tropical fish which inhabits off-shore coral reefs in Northern Australia and the Indo-Pacific region (Randall et al. 1990). The export of live coral trout comprises a major industry in these areas. Harvesting and processing procedures such as capture, handling, confinement and transportation frequently cause stress-induced reductions in stock quality that are attributable to the effects of secondary responses such as hyperlactacemia and tissue catabolism (Lowe et al. 1993). Management of the stress response, which has been achieved for certain cold-water species, will ameliorate these effects (Pickering 1992; Robertson et al. 1988). The characterization of the physiological response of fish to standard stressors is an important step in achieving improved management practices.

The aim of this study was to characterize primary and secondary responses of *P. leopardus* to capture and experimental stressors. The shallow water stressor was chosen as the experimental stressor, as it could be accurately controlled and repeated, irrespective of fish size or experimental environment (Carragher and Rees 1994; Einarsdottir and Nilssen 1996).

Materials and methods

In each experiment fish were subjected to one or more procedures (stressors) and subsequently blood-sampled at stages during or after the imposition of stress. Fish were sampled only once during each experiment, and 5 to 8 parameters were analysed on each blood sample. The majority of fish were used for more than one experiment, with a recovery period of at least 14 d.

Experiment 1

Experiment 1 examined the effects of capture, handling and transport related stressors on fish from wild populations of coral trout. Sixty-three fish (between 1 kg and 5 kg) were captured by angling with baited handlines on shallow reefs of the central Great Barrier Reef, Australia (17°45′ S; 146°30′ E). Fish were landed within 20 s of hooking and either blood sampled immediately (unstressed fish; time 0) or placed in groups of up to five into 70 l, aerated holding tanks and sampled at 15 min, 30 min, 1, 4, 12 or 72 h post-capture (9 fish per treatment). As in other studies, the time 0 samples were assumed not to be significantly different from resting or unstressed levels (Pankhurst and Sharples 1992).

Fish remained in the holding tanks during transport to the James Cook University Aquaculture Facility, Townsville, Australia. This involved 1.5 h sea travel and 3 h road travel. All sea transport was undertaken in calm weather (wind speed < 10 knots; waves $<$ 0.5 m). Samples up to 4 h were collected at sea, while the 12 and 72 h samples were collected at the facility.

Aquarium conditions

Thirty-six fish previously captured and sampled during Experiment 1 were used in subsequent experiments. The fish were accommodated individually in 70 l tanks. Each tank was supplied with approximately 1 l min^{-1} of water from a bio-filtered, recirculation system. The salinity, temperature and dissolved oxygen saturation of this system were maintained under conditions similar to those found in the natural habitat, 27–29 ppt, 28–30 °C and 80–90%, respectively. Fish were provided with a 12-h light/dark photoperiod and were undisturbed except for feeding with whole fish *ad libitum* once every three days.

Experiment 2

Experiment 2 investigated the effect of a standard reference stressor, exposure to shallow water, under controlled aquarium conditions. An external overflow outlet was lowered to reduce the water level over the course of approximately two min until the fish's back was exposed (6–12 cm deep depending on fish size) and the fish experienced difficulty remaining upright. Water aeration and flow were maintained. Once the water level was lowered, the fish were not disturbed otherwise until blood sampling. Fish were subjected to shallow water for 15, 30, 60, or 90 min (7 fish per treatment), followed by dip-net capture and immediate sampling. An additional 7 fish were caught and similarly sampled from tanks that did not have the water level lowered (unstressed fish).

Experiment 3

Experiment 3 examined the potential for recovery after acute exposure to shallow water stress. The water level was reduced in each tank as in Experiment 2. After 30 min the water level was allowed to return to its original depth over approximately 3 min by raising the overflow pipe. Fish were sampled at 30, 60,

150 or 240 min (7 fish per treatment) after the reduction in water level. As in Experiment 2, samples were collected at time 0 and considered as representing unstressed fish.

Experiment 4

Experiment 4 sought to determine if fish exhibited their maximum response to an initial acute stress or if they could respond further to successive stressors. The experimental design sought to emulate experiences of fish captured in the wild by commercial fishermen and subsequently kept alive for sale. Experimental fish were divided into five groups (7 fish per treatment). As in Experiment 2, samples were collected at time 0 and considered as representing unstressed fish. Fish in groups 2 to 5 were subjected to a 30 min shallow water stress as described in Experiment 2. A group of fish was then sampled (time 30 min: 1 stressor, group 2). The tanks of the fish in the three remaining treatments were refilled as described in Experiment 3 after experiencing this stressor. Fish in group 4 were allowed to recover for 210 min before sampling (time 240 min: 1 stressor). Fish in groups 3 and 5 were subjected to a second 30 min shallow water stressor commencing 30 min after the cessation of the first. Group 3 was sampled on completion of the second stressor (time 90 min: 2 stressors) and group 5 was allowed to recover for a further 210 min after the second stressor before sampling (time 300: 2 stressors).

Blood samples

Blood samples (2.5 ml) were collected from the caudal artery with a 22 gauge hypodermic needle. Fish were restrained in a wet foam cradle during this time. A blood smear was made immediately on a 1 mm glass microscope slide which was allowed to air-dry. One ml of blood was then allowed to clot for at least 2 h on ice, before centrifuging for 5 min at 10 000 g. The resulting serum was stored at -80 °C until assay. The remainder of the blood was stored on ice in a vial containing fluoride heparin to prevent clotting. Haematocrit and total leucocyte counts were undertaken the same day as sampling using heparinised blood. A 100 μl aliquot of whole blood was stored frozen at -20 °C for haemoglobin analysis. The remaining heparinised blood was centrifuged for 5 min at 10 000 g to prepare plasma that was either assayed immediately or stored frozen at -20 °C. The non-heparinised blood was similarly centrifuged and the serum stored frozen at -80 °C.

Cortisol

Plasma cortisol was determined by the radioimmunoassay (RIA) techniques as described by Pankhurst et al. (1992). The tracer used was [^{3}H]-cortisol (TRK407, Amersham, Australia) and AB1003 (Bioclin, United Kingdom) was used as antibody. Sample radioactivity was determined with a Wallac 1410 scintillation counter.

Glucose, lactate and haemoglobin

Plasma glucose and lactate and whole blood haemoglobin were assayed using test-kits adapted for use with a microplate reader. The glucose and lactate kits (nos. 716251 and 139084 respectively) were purchased from Boehringer-Mannheim (Castle Hill, Australia) and the haemoglobin kit (no. 525-A) was purchased from Sigma (Castle Hill, Australia).

Haematocrit

A heparinised microhaematocrit tube, 75 mm long with an inner bore of 1.2 mm, was filled approximately three-quarters with blood and centrifuged in a Beckman GS-6R centrifuge for 5 min at 3000 g. The haematocrit value (Hct) was calculated as in Dacie and Lewis (1984). Measurements were recorded to the nearest 0.5 mm. Changes in erythrocyte volume were calculated by subtracting the percentage change in haemoglobin from the percentage change in haematocrit during the same period.

Thrombocyte, lymphocyte and granulocyte count

Where measured, total leucocyte count was determined within 4 h of sampling by diluting heparinised blood 1:20 with Dacie's diluting fluid (Blaxhall and Daisley 1973). For this reason, it was not possible to measure leucocytes in the wild captured fish. The cell suspension was loaded onto an Improved Neubauer haemocytometer (Clay-Adams, Parsippany, U.S.A.) and the number of leucocytes per cubic millimetre of blood calculated (Rowley 1990). Differential leucocyte count was determined in blood smears stained with May-Grunwald and Giemsa (Gurr 1973). Slides were examined using a high power light microscope (100$\times$ magnification) and the leucocytes identified according to Ellis (1977). One hundred leucocytes were counted on each slide and classified as either thrombocytes, lymphocytes and granulocytes. Monocytes were grouped with granulocytes because, of

their functional and morphological similarities (Ellis 1977). Thrombocyte, lymphocyte and granulocyte counts were then calculated by multiplying the percentage of each leucocyte type by the total leucocyte count.

Statistical analyses

A $\log_{10}$ transformation was applied to the cortisol data, before proceeding with further analysis to overcome heteroscedasticity (Zar 1984). All other data were analysed without transformation. The data for each blood parameter were analysed by one-way analyses of variance (ANOVA) performed using the SPSS software package. A significant difference was considered to exist if $p < 0.05$. *Post hoc* comparisons of group means were made, using Tukey's HSD test. All data given in the text and figures are the arithmetic mean ± S.E.M. of untransformed data.

Results

Experiment 1

Fish sampled 30 min after capture from the wild had a significantly elevated ($p < 0.05$) cortisol concentration compared to fish sampled at time 0, although fish sampled after 15 min did not have this concentration (Figure 1a). Cortisol concentration continued to increase until 4 h post-capture when the highest observed concentration was 70.5 ± 7.8 ng ml^{-1}. Cortisol levels significantly decreased ($p < 0.05$) from maximum concentrations by 72 h post-capture, but recovery to time 0 levels was not observed within the experimental period.

Glucose and lactate responded rapidly to capture stress. By 15 min post-capture, both glucose (Figure 1b) and lactate (Figure 1c) concentrations were significantly higher ($p < 0.05$) than at time 0. Peak concentrations were observed between 30 min and 1 h for glucose (maximum value $= 7.56 \pm 0.35$ mmol l^{-1} at 60 min) and between 15 min and 60 min for lactate (maximum value $= 4.14 \pm 0.52$ mmol l^{-1} at 30 min). Glucose and lactate concentrations were not different to time 0 levels by 72 and 12 h post-capture, respectively.

Capture stress caused significant increases ($p < 0.05$) in both Hb (Figure 1d) and Hct (Figure 1e) after 30 min and 15 min, respectively. At 30 min post-capture Hb had increased from 6.47 ± 0.23 to 9.28 ± 0.29 g l^{-1} indicating an increase in erythrocytes of 43%. At the same time Hct had increased by 53% from 29.0 ± 1.0 to 44.5 ± 1.3% and erythrocyte volume by 10%. These data indicate that both erythrocyte release and swelling occur in response to stress associated with capture, handling and transport. At 4 h post-capture both Hb and Hct levels were not significantly different ($p > 0.05$) to time 0 levels.

Experiment 2

Cortisol levels were significantly elevated ($p < 0.05$) after 60 min of continuous shallow water stress (Figure 2a) and the maximum observed concentration of 47.2 ± 9.7 ng ml^{-1} occurred at 90 min.

Glucose concentrations were significantly elevated in stressed fish ($p < 0.05$) relative to those of unstressed (time 0) fish after 15 min (Figure 2b). The maximum glucose concentration of 6.75 ± 0.42 mmol l^{-1} was observed at 60 min. Despite continued shallow water stress thereafter, glucose concentrations declined and were not significantly different ($p > 0.05$) from those of unstressed fish at 60 or 90 min.

Lactate concentration increased slowly in response to continuous shallow water stress but was not significantly different ($p > 0.05$) to unstressed fish until 90 min (Figure 2c). The maximum observed concentration was 3.97 ± 0.81 mmol l^{-1}.

Haemoglobin (Figure 2d) and Hct (Figure 2e) increased rapidly in stressed fish and were significantly different ($p < 0.05$) to those of unstressed fish after 30 and 15 min, respectively. The highest observed level of Hb was 8.95 ± 0.33 g l^{-1} at 60 min and of Hct was 46.4 ± 1.3% at 30 min. After 30 min erythrocyte number and volume had increased by approximately 27 and 33%, respectively. After 90 min both Hb and Hct had begun to decline and Hb was not significantly different ($p > 0.05$) to unstressed levels at this time.

Significant changes ($p < 0.05$) in thrombocyte (Figure 2f), lymphocyte (Figure 2g) and granulocyte (Figure 2h) count were detected after continuous exposure to shallow water. Thrombocyte counts in stressed fish was significantly higher ($p < 0.05$) than unstressed fish after 60 min. Lymphocyte and granulocyte count in stressed fish were significantly lower ($p < 0.05$) than unstressed fish at both 60 and 90 min.

Experiment 3

A single 30 min episode of shallow water stress elicited a sustained cortisol response with no sign of

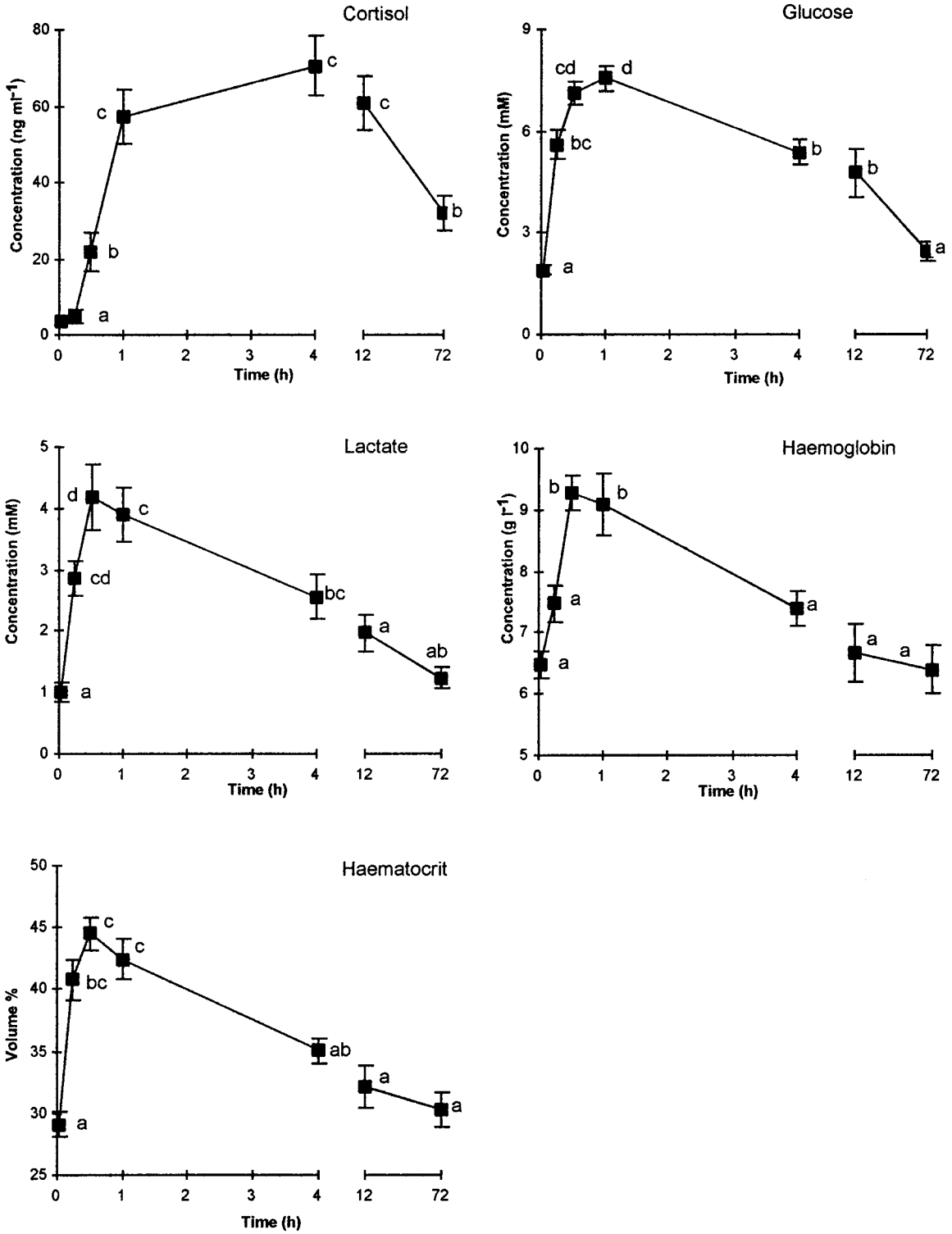

Figure 1. Changes with time in circulating levels of cortisol, glucose, lactate, haemoglobin and haematocrit after capture by angling from the wild and transport. Data are presented as mean ± SE for 9 fish at each sampling time. Values with the same letters are not significantly different ($p < 0.05$).

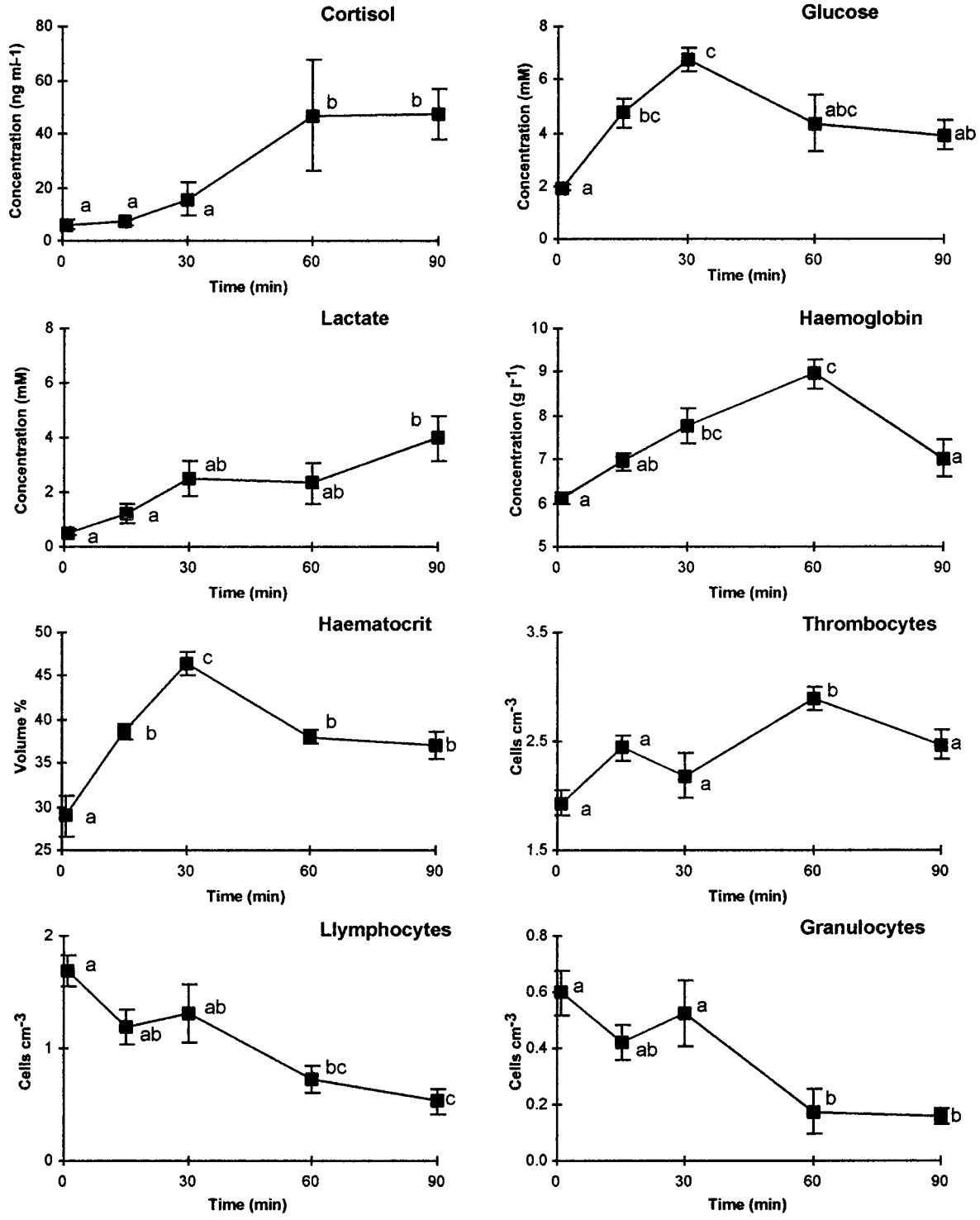

Figure 2. Circulating levels of cortisol, glucose, lactate, haemoglobin, haematocrit, thrombocytes, lymphocytes and granulocytes after exposure to continuous shallow water stress. Data are presented as mean ± SE for 7 fish at each sampling time. Values with different letters are significantly different to each other ($p < 0.05$).

recovery after 4 h (Figure 3a). Observed increases were significantly higher ($p < 0.05$) than time 0 values after 60 min when maximum concentrations were 18.3 ± 7.8 ng ml^{-1}. Cortisol concentrations declined slightly thereafter, but were still significantly higher ($p < 0.05$) than unstressed values 210 min after termination of the stressor.

In comparison to levels in unstressed fish, glucose (Figure 3b) and lactate (Figure 3c) concentrations were significantly elevated ($p < 0.05$), immediately after the stress period (30 min). However, glucose and lactate levels recovered and were not significantly different ($p > 0.05$) from unstressed levels 120 and 30 min respectively after the stressor was removed.

Haemoglobin concentration increased to 6.85 ± 0.20 g l^{-1} after 30 min of shallow water (Figure 3d), although this value was not significantly different ($p > 0.05$) to unstressed levels. At 60, 150 and 240 min Hb concentration had returned to unstressed levels. Similarly, Hct rose rapidly with 30 min of shallow water stress (Figure 3e) thereafter returning to unstressed levels by 150 min.

At 150-min thrombocyte count was significantly higher ($p < 0.05$) than that at time 0 (Figure 3f). However thrombocyte count recovered and was not significantly different ($p < 0.05$) from unstressed levels at 240 min. No significant changes ($p > 0.05$) were observed in either lymphocyte count or granulocyte count within the 4 h experimental period.

Experiment 4

A single 30-min episode of shallow water stress caused a sustained increase in cortisol concentration that was significantly different ($p < 0.05$) to unstressed fish (group 1) at both 30 (group 2) and 240 min (group 4) (Figure 4a). There was no significant difference ($p > 0.05$) in cortisol concentration between fish sampled immediately after one (group 2, time 30 min: 1 stressor) or two (group 3, time 90 min: 2 stressors) episodes of stress. However, 210 min after cessation of the second 30 min stress episode, cortisol concentration had increased further and was significantly higher ($p < 0.05$) than all preceding time points (group 5) (Figure 4a). Cortisol concentration was 11.5 ± 1.1 ng ml^{-1} after the first episode of stress and 51.7 ± 13.2 ng ml^{-1} after the second. Thus, two sequential shallow water episodes appear to provoke a cumulative cortisol response.

Relative to unstressed values (group 1), glucose (Figure 4b), Hb (Figure 4d) and Hct (Figure 4e) were significantly increased after either one (group 2, time 30 min: 1 stressor) or two (group 3, time 90 min: 2 stressors) episodes of shallow water stress. As they did not differ significantly between the two treatments (groups 2 and 3), there appear to be no additional effects of the second episode of stress on these parameters. Each of these parameters had returned to unstressed values by 210 min after the last stressor experienced (time 240 min: 1 stressor or time 300 min: 2 stressors).

Lactate (Figure 4c) was significantly higher in fish that experienced a single 30 min stress (group 2, time 30 min: 1 stressor) than in unstressed fish (group 1) and was further increased by a second 30 min shallow water stress (group 3, time 90 min: 2 stressors), thus indicating an additive effect of the stressors. In fish that experienced both one and two stressors, lactate had returned to unstressed levels by 210 min after the last stressor experienced (time 240 min: 1 stressor and time 300 min: 2 stressors).

Discussion

The physiological response of coral trout to capture, handling and transport related stressors and a controlled stressor was, in general, similar to that reported for temperate or cold-water species (Haux et al. 1985; Pankhurst and Sharples 1992; Woodward and Strange 1987). However, the data obtained for coral trout indicate that high circulating levels of glucose and cortisol are apparently not connected in this species. A clear depression of cellular based immunity by stress was also apparent.

Mean plasma cortisol concentrations in fish sampled immediately after capture from the wild of less than 5 ng ml^{-1} were similar to values reported for other species sampled under similar conditions (Carragher and Rees 1994; Pankhurst and Sharples 1992). The cortisol response in coral trout (*P. leopardus*) is chronic in nature as it is in a variety of other species (Barton and Iwama 1991; Lowe and Wells 1996; Pankhurst and Sharples 1992). Circulating cortisol showed a latent period of 15 to 30 min after capture from the wild or 30 to 60 min of continuous shallow water stress occurred before increasing. Maximum values observed during this experiment were lower than those recorded in other species for these (Carragher and Rees 1994; Einarsdottir and Nilssen 1996) or similar stressors (Robertson et al. 1988; Specker and Schreck 1980) being 70.5 ± 7.8 ng ml^{-1} fol-

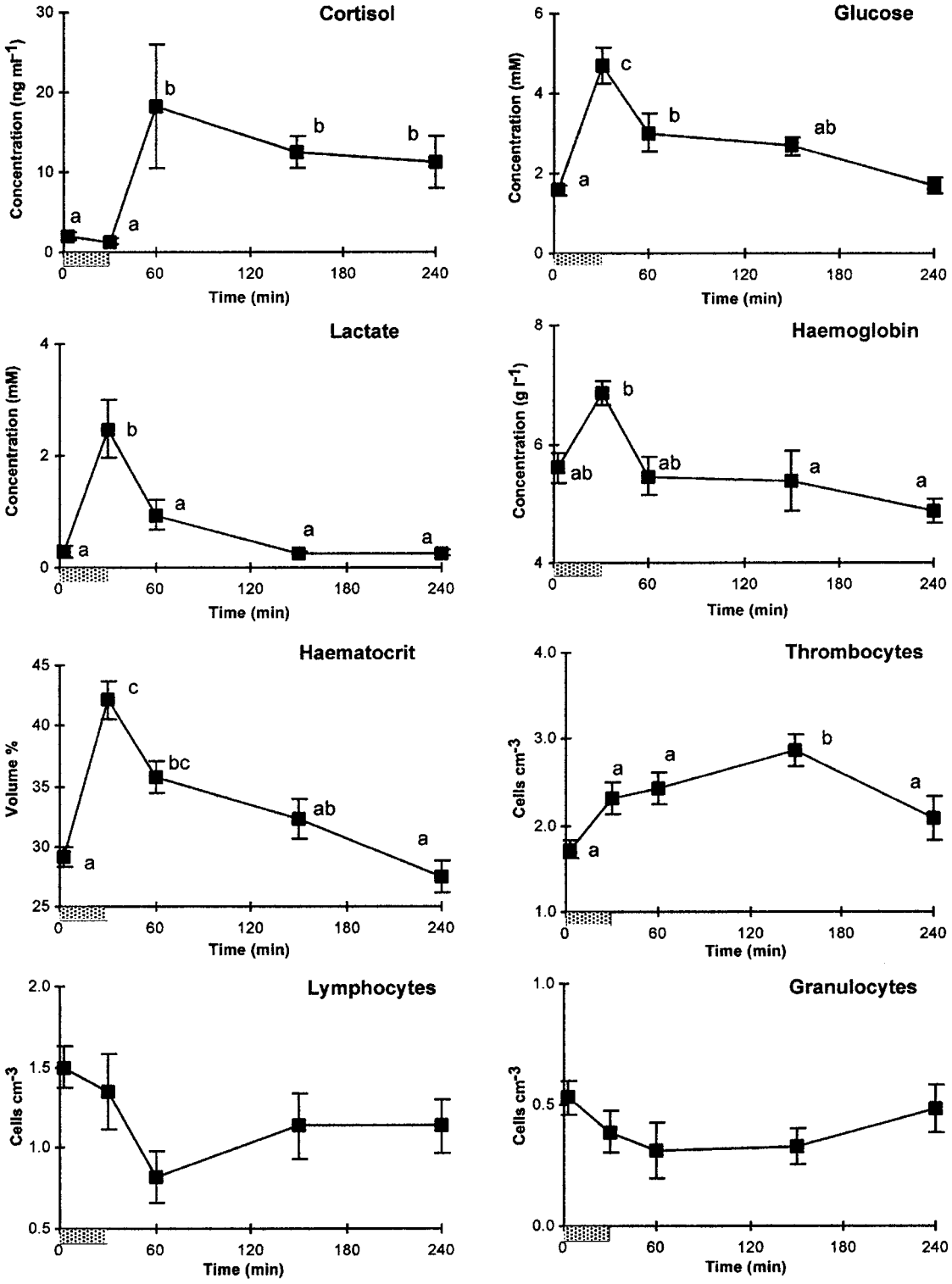

Figure 3. Changes with time in circulating levels of cortisol, glucose, lactate, haemoglobin, haematocrit, thrombocytes, lymphocytes and granulocytes after exposure to a 30 min episode of shallow water stress. The stippled box indicates the period of the stressor. Data are presented as mean ± SE for 7 fish at each sampling time. Values with different letters are significantly different to each other ($p < 0.05$).

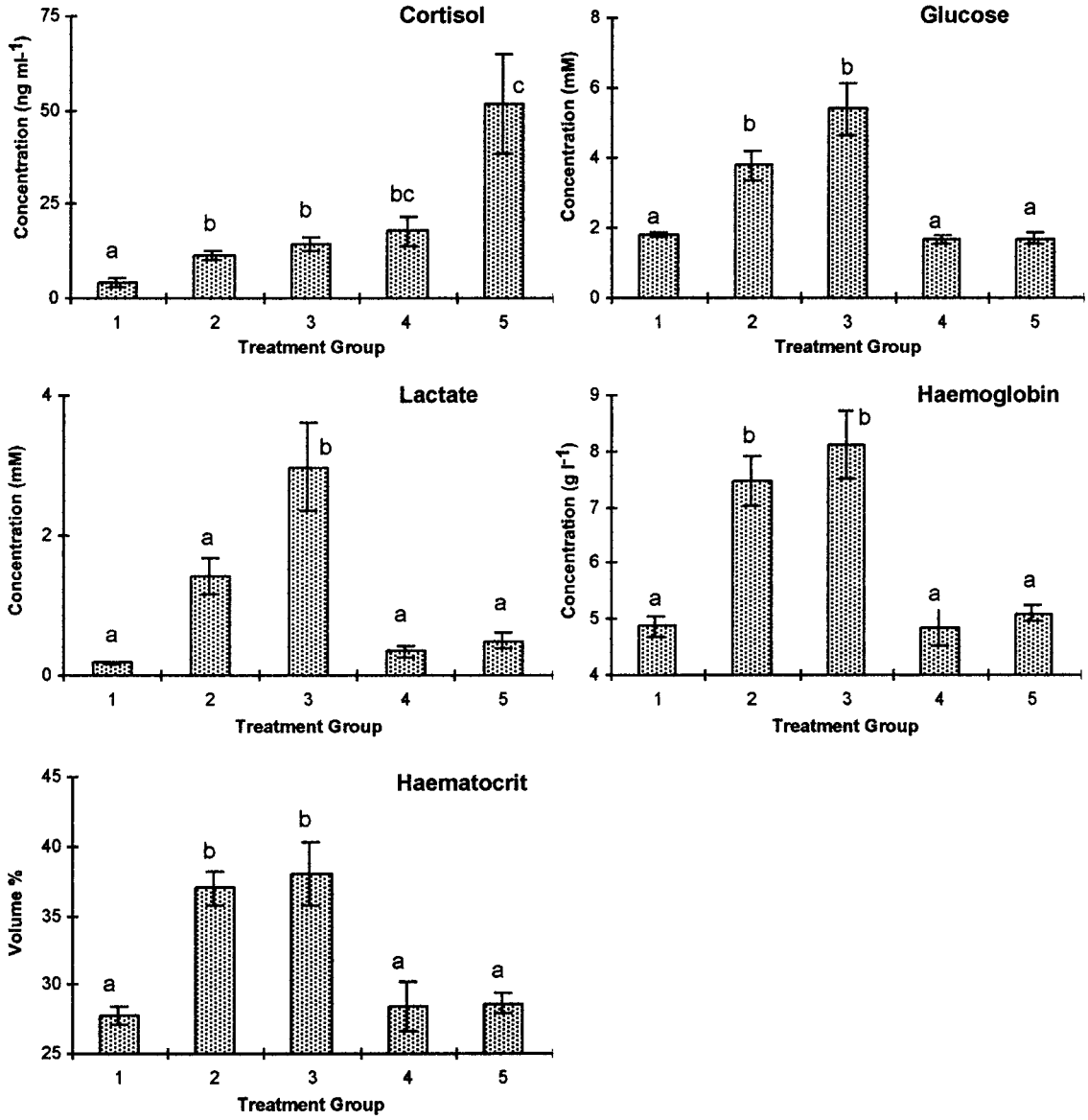

Figure 4. Circulating levels of cortisol, glucose, lactate, haemoglobin and haematocrit after no stressor (Group 1), a single 30 min episode of shallow water stress (Group 2), two 30 min episodes of shallow water stress separated by a 30 min recovery period (Group 3), a single 30 min episode of shallow water stress followed by a 210 min recovery period (Group 4), or two 30 min episodes of shallow water stress separated by a 30 min recovery period and followed by a 210 min recovery period (Group 5). Data are presented as mean ± SE for 5–7 fish at each sampling time. Values with different letters are significantly different to each other ($p < 0.05$).

lowing wild capture or 47.2 ± 9.7 ng ml^{-1} during shallow water stress. Many other species subjected to prolonged or intense stressors elicit cortisol responses in the order of 100–500 ng ml^{-1} (Barton and Iwama 1991). The slower and lesser response to the controlled stressor compared with wild capture, handling and transport may reflect the relative intensity of this stressor.

Recovery of cortisol to unstressed levels was slow with cortisol remaining elevated throughout the sampling period of 3 d in wild captured coral trout or 210 min following a shallow water stressor. Cortisol concentrations were maximal 30 min after termination of the stressor and a decline in mean cortisol concentration was observed thereafter. Recovery of cortisol levels in salmonid species exposed to acute stress is

in the range of 4 to 24 h (Barton and Schreck 1987; Pickering et al. 1982) although cortisol in seabream, *S. aurata*, returns to basal levels within 2 h of a 3 min air exposure (Arends et al. 1999).

When coral trout experienced a single stressor, plasma cortisol concentration rose to a plateau, rather than increasing indefinitely. Such a response has previously been attributed to the negative feedback of cortisol on the hypothalamus, which results in suppressed adrenocorticotrophic hormone (ACTH) release from the pituitary (Barton and Iwama, 1991). Barton et al. (1986) suggest that multiple stressors evoke cumulative responses, because this feedback mechanism operates less effectively when fish are allowed a short recovery period between disturbances. The data obtained in this study of coral trout using these recovery periods do not support this contention. The value observed as a result of two episodes of 30 min separated by a recovery of 30 min (51.7 ± 13.1 ng ml^{-1}) was similar to that found in fish experiencing 60 min of continuous stress (46.7 ± 20.9 ng ml^{-1}).

Thus HPI activation in coral trout is generally smaller in magnitude, but similar in duration, to other teleosts.

Hyperglycaemia is a commonly reported stress response in fish (Braley and Anderson 1992; Specker and Schreck 1980). Plasma glucose concentrations increased in coral trout within 15 min of capture from the wild or in response to shallow water stress. Unlike cortisol, however, a maximum concentration was reached 30 min after initiation of the stressor and blood glucose was not different to unstressed levels by 60 min in animals that were subject to continued shallow water stress and 150 min in those that were allowed to recover. Furthermore, *P. leopardus* did not significantly increase blood glucose concentrations when exposed to a second episode of stress relative to those that experienced only one episode. It has been previously considered that catecholamines induce a release of glucose into the blood that is subsequently maintained by cortisol (Pickering et al. 1982). In other species, acute stress causes an increase in blood metabolites which continues for at least as long as elevated cortisol levels persist (Barton et al. 1986; Pickering et al. 1982; Vijayan and Leatherland 1989). In the present study, elevation of blood glucose was transient, occurring before the onset of increased blood cortisol and recovering to unstressed levels despite continued high circulating concentrations of cortisol. The decline in blood glucose may be a result of glucose exhaustion or increased glucose utilisation in these animals, but wild captured fish maintain elevated blood glucose for up to 72 h after capture. Thus, glucose reserves appear capable of being released for much longer than observed and at levels greater than the immediate metabolic demand. Whilst glucose turnover rate measurements under the conditions experienced by the fish in this study are required to allow definitive conclusions, it is apparent that blood glucose concentration in acutely stressed coral trout does not follow circulating levels of cortisol.

Capture stress provoked a rapid increase in lactate, which stabilised after 30 min, but did not decline to unstressed levels until 12 h post-capture. Lactate is produced by anaerobic metabolism in the white muscle under conditions of hypoxia or strenuous exercise (Driedzic and Kiceniuk 1976). It is likely that both these situations prevailed during the present study as a result of emersion during hook removal and swim bladder deflation and struggling of fish during capture and subsequent hook removal. Consequently, it is not surprising that blood lactate levels were markedly elevated during the post-capture period. Increases in plasma lactate during both acute and chronic shallow water stress is also likely to occur, due to exercise associated with struggling since normal water flow and aeration were maintained throughout the stress period. The effect of a second episode of shallow water was to further increase circulating lactate. The concentrations of lactate observed during this study were similar to those of other species exposed to acute experimental stressors similar to the ones used here (Carragher and Rees 1994; Waring et al. 1992).

The short recovery time of Hb and Hct observed in this study is typical of catecholamine-induced responses to acute stress and has previously been described (Lowe and Wells 1996; Soivio and Oikari 1976). Hct was significantly elevated at 15 min and maximal at 30 min after capture from the wild and in response to shallow water stress, while blood Hb reached maximal levels at 30 to 60 min after the onset of the stressor. In wild captured fish, erythrocyte concentration (as indicated by Hb concentration) increased by approximately 43% at 30 min after capture while erythrocyte volume increased by approximately 10%. This is similar to values reported for yellowtail (*Seriola quinqueradiata*) sampled immediately after 5 min severe exercise (Yamamoto et al. 1980). Recovery of blood Hb content was apparent by 30 min after removal of the shallow water stressor or by 90 min when the fish were subjected to continuing shallow

water. Recovery of blood Hct to unstressed levels was apparent by 120 min after the removal of shallow water stress. Repeated episodes of shallow water stress did not further increase Hb or Hct levels over those observed after a single episode and did not appear to delay recovery to unstressed levels.

Coral trout showed a significant response in immune function to stress. The concentration of circulating thrombocytes increased in response to shallow water stress. Thrombocytes are an important component of the blood clotting mechanism in fish (Houston 1990) and rainbow trout blood coagulates more rapidly after stress, when it contains greater concentrations of thrombocytes (Cassillas and Smith 1977). Cassillas and Smith (1977) suggested this observation was a catecholamine response, serving to prepare the fish for blood loss which often occurs during stressful situations. That thrombocyte numbers returned to unstressed levels 210 min after cessation of the stressor even though cortisol remained elevated at this time, supports the hypothesis that it is a catecholaminergic response.

Lymphocyte and granulocyte numbers declined in coral trout in response to shallow water stress, and by 60 min were significantly lower than unstressed values. The ability of lymphocytes from killifish (*Fundulus heteroclitus*), exposed to chronic confinement stress to proliferate in response to mitogenic stimuli, was suppressed (Miller and Tripp 1982). Furthermore, lymphocytes from channel catfish (*Ictalurus punctatus*) exposed to handling stress were either damaged or functionally impaired (Ellsaesser and Clem 1986) and cortisol induced loss of lymphocytes in brown trout (*Salmo trutta*) (Pickering 1984).

Mechanisms of innate immunity, such as that afforded by granulocytes, are of great importance in fish (Sunyer and Tort 1995). Thus, the decrease in the concentration of lymphocytes and granulocytes observed in the present study, would be likely to result in a significantly enhanced susceptibility to disease.

This study has characterized the response of coral trout to two types of stressor. As in other species, activation of the SC system in coral trout causes increases in the availability of carbohydrate substrates, the concentration of erythrocytes, and the affinity of Hb for oxygen. The net result would be an increase in the availability of energy and oxygen for immediate muscle activity. In contrast, the HPI axis is stimulated in response to long term stressors. The subsequent release of cortisol is hypothesized to maintain the high levels of circulating energy sources provoked by catecholamines, thereby sustaining the response of a fish to a stressor (Leach and Taylor 1980; van der Boon 1991). However, this investigation failed to show a connection between circulating glucose and cortisol levels in coral trout.

The findings of the present study also have several important commercial implications. Stress-induced lactate production can cause, among other things, significant reductions in flesh quality, by lowering tissue pH and promoting the onset of rigor mortis (Elvevoll et al. 1996; Shahidi and Botta 1994). Similarly, stress appears to cause a suppression of cell-mediated immunity by reducing numbers of lymphocytes and granulocytes in circulation. Clearly, commercial fishing and aquaculture practices that minimize physiological stress responses will enhance the health, flesh quality and survival of fish. Secondly, coral trout responded quickly to stressors with maximal values of blood parameters generally lower than those reported for cultured species (Barton and Iwama 1991; Davis and Parker 1986). They also appear to recover rapidly after acute stress and acclimate well to aquarium conditions after capture. These characteristics suggest that coral trout are capable of tolerating stressors found in aquaculture and may therefore be appropriate for culture in the future.

Acknowledgements

This work was supported by grants from JCU to TAA and from the Co-operative Research Centre for Sustainable Development of the Great Barrier Reef to AJF.

References

Arends, R.J., Mancera, J.M., Muñoz, J.L., Wendelaar Bonga, S.E. and Flik, G. 1999. The stress response of the gilthead seabream (*Sparus aurata* L.) to air exposure and confinement. J. Endocrinol. 163: 149–157.

Barton, B.A. and Iwama, G.K. 1991. Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Annu. Rev. Fish. Dis. 1: 3–26.

Barton, B.A. and Peter, R.E. 1982. Plasma cortisol stress response in fingerling rainbow trout, *Salmo gairdneri*, to various transport conditions, anaesthesia and cold-shock. J. Fish. Biol. 20: 39–51.

Barton, B.A. and Schreck, C.B. 1987a. Influence of acclimation temperature on interrenal and carbohydrate stress responses in juvenile chinook salmon (*Oncorhynchus tshawytscha*). Aquaculture 62: 299–310.

Barton, B.A., Schreck, C.B. and Sigismondi, L.A. 1986. Multiple acute disturbances evoke cumulative physiological stress

responses in juvenile chinook salmon. Trans. Am. Fish. Soc. 115: 245–251.

Blaxhall, P.C. and Daisley, K.W. 1973 Routine haematological methods for use with fish blood. J. Fish Biol. 5: 771–781.

Braley, H. and Anderson, T.A. 1992. Changes in blood metabolite concentrations in response to repeated capture, anaesthesia and blood sampling in the golden perch, *Macquaria ambigua*. Comp. Biochem. Physiol. 103A: 445–450.

Carragher, J.F. and Rees, C.M. 1994. Primary and secondary stress responses in golden perch, *Macquaria ambigua*. Comp. Biochem. Physiol. 107A: 49–56.

Cassilas, E. and Smith, L.S. 1977. Effects of stress on blood coagulation and haematology in rainbow trout (*Salmo gairdneri*). J. Fish Biol. 10: 481–491.

Dacie, J.V. and Lewis, S. 1984. Practical Haematology. Churchill Livingstone, Edinburgh.

Davis, K.B. and Parker, N.C. 1986. Plasma corticosteroid stress response of fourteen species of warmwater fish to transportation. Trans. Am. Fish. Soc. 115: 495–499.

Davis, K.B. and Parker, N.C. 1990. Physiological stress in striped bass: effect of acclimation temperature. Aquaculture 91: 349–358.

Driedzic, W.R. and Kiceniuk, J.W. 1976. Blood lactate levels in free-swimming rainbow trout (*Salmo gairdneri*) before and after strenuous excercise resulting in fatigue. J. Fish. Res. Board Can. 33: 173–176.

Ellis, A.E. 1977. The leucocytes of fish: a review. J. Fish Biol. 11: 453–491.

Ellsaesser, C.F. and Clem, L.W. 1986. Haematological and immunological changes in channel catfish stressed by handling and transport. J. Fish. Biol. 28: 511–521.

Elvevoll, E.O., Sorenson, N.K., Osterud, B., Ofstad, R. and Martinez, I. 1996. Processing of Marine Foods. Meat Sci. 43: 265–275.

Einarsdottir, I.E. and Nilssen, K.J. 1996. Stresss responses of Atlantic salmon (*Salmo salar*) elicited by water level reduction in rearing tanks. Fish Physiol. Biochem. 15: 395–400.

Gurr, E. 1973. Biological Staining Methods. Searle Diagnostic, Buckinghamshire.

Haux, C., Sjobeck, M.L. and Larsson, A. 1985. Physiological stress responses in a wild fish population of perch (*Perca fluviatilis*) after capture and during subsequent recovery. Mar. Environ. Res. 15: 77–95.

Houston, A.H. 1990. Blood and circulation. *In*: Methods for Fish Biology. pp. 273–334. Edited by C.B. Schreck and P.B. Moyle. American Fisheries Society, Bethesda.

Leach, G.J. and Taylor, M.H. 1980. The role of cortisol in stress-induced metabolic changes in *Fundulus heteroclitus*. Gen. Comp. Endocrinol. 42: 217–227.

Lowe, T.E., Ryder, J.M., Carragher, J.F. and Wells, R.M.G. 1993. Flesh quality in snapper, *Pagrus auratus*, affected by capture stress. J. Food Sci. 58: 770–773.

Lowe, T.E. and Wells, R.M.G. 1996. Primary and secondary stress responses to line capture in the blue mao mao. J. Fish Biol. 49: 287–300.

Miller, N.W. and Tripp, M.R. 1982. The effect of captivity on the immune response of the killifish, *Fundulus heteroclitus*. J. Fish. Biol. 20: 301–308.

Pankhurst, N.W. and Sharples, D.F. 1992. Effects of capture and confinement on plasma cortisol concentrations in the snapper, *Pagrus auratus*. Aust. J. Mar. Freshwater Res. 43: 345–356.

Pankhurst, N.W., Wells, R.M.G. and Carragher, J.F. 1992. Effects of stress on plasma cortisol levels and blood viscosity in blue mao mao, *Scorpis violaceus* (Hutton), a marine teleost. Comp. Biochem. Physiol. 101A: 335–339.

Pickering, A.D. 1984. Cortisol-induced lymphocytopenia in brown trout, *Salmo trutta*. Gen. Comp. Endocrinol. 53: 252–259.

Pickering, A.D. 1992. Rainbow trout husbandry: management of the stress response. Aquaculture. 100: 125–139.

Pickering, A.D., Pottinger, T.G. and Christie, P. 1982. Recovery of the brown trout, *Salmo trutta*, from accute handling stress: a time-course study. J. Fish. Biol. 20: 229–244.

Randall, J.E., Allen, G.R. and Steene, R.C. 1990. Fishes of the Great Barrier Reef and Coral Sea. Crawford House Press, Bathurst.

Robertson, L., Thomas, P. and Arnold, C.R. 1988. Plasma cortisol and secondary stress responses of cultured red drum (*Sciaenops ocellatus*) to several transportation procedures. Aquaculture 68: 115–130.

Rowley, A.F. 1990. Collection, separation and identification of fish leucocytes. *In*: Techniques in Fish Immunology. Edited by J.S. Stolen, T.C. Fletcher, D.P. Anderson, B.S. Roberson and W.B. van Muiswinkel. SOS Publications, New Jersey.

Schreck. C.B. 1982. Stress and rearing of salmonids. Aquaculture 28: 241–249.

Shahidi, F. and Botta, J.R. 1994. Seafoods: Chemistry, Processing Technology and Quality. Blackie Academic and Professional,London.

Soivio, A. and Oikari, A. 1976. Haematological effects of stress on a teleost, *Esox lucius*. J. Fish. Biol. 8: 397–411.

Specker, J.L. and Schreck, C.B. 1980. Stress responses to transportation and fitness for marine survival in coho salmon (*Oncorhynchus kisutch*) smolts. Can. J. Fish. Aquat. Sci. 37: 765–769.

Sumpter, J.P. 1991. The stress response and its consequences in cultured fish. Bull. Inst. Zool. 16: 229–236.

Sunyer, J.O. and Tort, L. 1995. Natural hemolytic and bactericidal activities of sea bream *Sparus auratus* serum are effected by the alternative complement pathway. Vet. Immunol. Immunopathol. 45: 333–345.

van der Boon, J., van den Thillart, G.E.E.J.M. and Addink, A.D.F. 1991. The effect of cortisol administration on intermediary metabolism in teleost fish. Comp. Biochem. Physiol. 100(1): 47–53.

Vijayan, M.M. and Leatherland, J.F. 1989. Cortisol-induced changes in plasma glucose, protein, and thyroid hormone levels, and liver glycogen content of coho salmon (*Oncorhynchus kisutch*). Can. J. Zool. 67: 2746–50.

Waring, C.P., Stagg, R.M. and Poxton, M.G. 1992. The effects of handling on flounder (*Platichthys flesus*) and Atlantic salmon (*Salmo salar*). J. Fish. Biol. 41: 131–144.

Wedemeyer, G. and McLeay, D.J. 1981. Methods for determining the tolerance of fishes to environmental stressors. *In*: Stress and Fish. Edited by A.D. Pickering. Academic Press, London.

Wendelaar Bonga, S.E. 1997. The stress response in fish. Physiol. Rev. 77: 591–625.

Woodward, C.C. and Strange, R.J. 1987. Physiological stress responses in wild and hatchery-reared rainbow trout. Trans. Am. Fish. Soc. 116: 574–579.

Yamamoto, K-I., Itazawa, Y. and Kobayashi, H. 1980. Supply of erythrocytes into the circulating blood from the spleen of excercised fish. Comp. Biochem. Physiol. 65A: 5–11.

Zar, J.H. 1984 Biostatistical Analysis. Prentice-Hall Publishers, London.